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(54) Title: SPECIFIC ANTIBODIES TO AMYLOID β PEPTIDE, PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The invention relates to methods of treating a subject having Alzheimer's Disease, comprising the step of administering an antibody molecule which is targeted to β amyloid peptide or to fragment thereof. In another embodiment the invention relates to methods of treating a disease or a disorder, characterized by amyloid beta deposition. In another embodiment, the invention relates to an antibody molecule, which is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide and to a pharmaceutical composition thereof. In another embodiment, the invention relates to an antibody molecule, which is targeted to the free C or N-terminus of a N-and/or C-terminus truncated amyloid β peptide fragment.

Specific Antibodies to Amyloid β Peptide, Pharmaceutical Compositions and Methods of Use Thereof

FIELD OF THE INVENTION

The invention relates to methods of treating a subject having Alzheimer's Disease, comprising the step of administering an antibody molecule which is targeted to β amyloid peptide or to fragment thereof. In another embodiment, the invention relates to methods of treating a disease or a disorder, characterized by amyloid beta deposition. In another embodiment, the invention relates to an antibody molecule, which is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide, or fragment thereof, and to a pharmaceutical composition comprising the same.

BACKGROUND OF THE INVENTION

A major histopathological hallmark of Alzheimer's Disease (AD) is the presence of amyloid deposits within neuritic and diffuse plaques in the parenchyma of the amygdala, hippocampus and neocortex (Glenner and Wong, 1984; Masters et al., 1985; Sisodia and Price, 1995). Amyloid is a generic term that describes fibrillar aggregates that have a common β -pleated structure. These aggregates exhibit birefringent properties in the presence of Congo red and polarized light (Glenner and Wong, 1984). The diffuse plaque is thought to be relatively benign in contrast to the neuritic plaque which appears to be strongly correlated with reactive and degenerative processes (Dickson et al., 1988; Tagliavini et al., 1988; Yamaguchi et al., 1989; Yamaguchi et al., 1992). One of the principal components of neuritic plaques is a 42 amino acid residue amyloid - β (Aβ) peptide (Miller et al., 1993; Roher et al., 1993) that is derived from the much larger β -amyloid precursor protein. β APP (or APP) (Kang et al., 1987). Aβ 1-42 is produced less abundantly than the 1- 40 Aβ peptide (Haass et al., 1992; Seubert et al., 1992), but the preferential deposition of Aβ 1-42 results from the fact that this COOH-extended

form is more insoluble than 1-40 A β and is more prone to aggregate and form anti-parallel β- pleated sheets (Joachim et al., 1989; Halverson et al., 1990; Barrow et al., 1992; Burdick et al., 1992; Fabian et al., 1994). Aβ 1-42 can seed the aggregation of Aβ 1-40 (Jarrett and Lansbury 1993).

The APP gene was sequenced and found to be encoded on chromosome 21 (Kang al., 1987). Expression of the APP gene generates several Aβ -containing isoforms of 695, 751 and 770 amino acids, with the latter two APPs containing a domain that shares structural and functional homologies with Kunitz serine protease inhibitors (Kang et al., 1987; Kitaguchi et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Konig et al., 1992). The functions of APP in the nervous system remain to be defined, although there is increasing evidence that APP has a role in mediating adhesion and growth of neurons (Schubert et al., 1989; Saitoh et al., 1994; Roch, 1995) and possibly in a G protein-linked signal transduction pathway (Nishimoto et al., 1993). In cultured cells, APPs mature through the constitutive secretory pathway (Weidemann et al., 1989; Haass et al., 1992; Sisodia 1992) and some cell-surface-bound APPs are cleaved within the Aβ domain by an enzyme, designated α - secretase, (Esch et al., 1990), an event that precludes Aβ amyloidogenesis. Several studies have delineated two additional pathways of APP processing that are both amyloidogenic: first an endosomal/lysosomal pathway generates a complex set of APP- related membrane-bound fragments, some of which contain the entire Aβ sequence (Haass et al., 1992; Golde et al., 1992); and second, by mechanisms that are not fully understood, Aβ 1-40 is secreted into the conditioned medium and is present in cerebrospinal fluid in vivo (Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992; Busciglio et al., 1993). Lysosomal degradation is no longer thought to contribute significantly to the production of A β (Sisodia and Price 1995). The proteolytic enzymes responsible for the cleavages at the NH2 and COOH termini of A β are termed β (BACE) and γ secretase, respectively. Until recently, it was generally believed that Aβ is generated by aberrant metabolism of the precursor. The presence. however, of Aβ in conditioned medium of a wide variety of cells in culture and in human cerebrospinal fluid suggest that Aβ is produced as a normal function of cells.

The main focus of researchers and the principal aim of those associated with drug development for AD is to reduce the amount of Aβ deposits in the central nervous system (CNS). These activities fall into several general areas: factors affecting the production of Aβ, the clearance of Aβ, and preventing the formation of insoluble Aβ fibrils. Another therapeutic goal is to reduce inflammatory responses evoked by Aβ neurotoxicity.

Given that neurotoxicity appears to be associated with β -pleated aggregates of Aβ, one therapeutic approach is to inhibit or retard Aβ -aggregation. The advantage of this approach is that the intracellular mechanisms triggering the overproduction of Aβ or the effects induced intracellularly by Aβ need not be well understood. Various agents that bind to Aβ are capable of inhibiting Aβ neurotoxicity in vitro, for example, the Aβ -binding dye, Congo Red, completely inhibits Aβ -induced toxicity in cultured neurons (Yankner et al., 1995). Similarly, the antibiotic rifampacin also prevents Aβ aggregation and subsequent neurotoxicity (Tomiyama et al., 1994). Other compounds are under development as inhibitors of this process either by binding Aβ directly, e.g., hexadecyl-N-methylpiperidinium (HMP) bromide (Wood et al., 1996), or by preventing the interaction of Aβ with other molecules that contribute to the formation of Aβ deposition. An example of such a molecule is heparan sulfate or the heparan sulfate proteoglycan, perlecan, which has been identified in all amyloids and is implicated in the earliest stages of inflammation associated amyloid induction.

Heparan sulfate interacts with the Aβ peptide and imparts characteristic secondary and tertiary amyloid structural features. Recently, small molecule anionic sulfates have been shown to interfere with this reaction to prevent or arrest amyloidogenesis (Kisilevsky, 199S), although it is not evident whether these compounds will enter the CNS. A peptide based on the sequence of the perlecan- binding domain appears to inhibit the interaction between Aβ and perlecan, and the ability of Aβ -derived peptides to inhibit self-polymerization is being explored as a potential lead to developing therapeutic treatments for AD. The effectiveness of these compounds in

vivo, however, is likely to be modest for a number of reasons, most notably the need for chronic penetration of the blood brain barrier.

An alternative to a peptide-based approach is to elucidate the cellular mechanism of Aβ neurotoxicity and develop therapeutics aimed at those cellular targets. The focus has been on controlling calcium dysfunction of free radical mediated neuronal damage. It has been postulated that Aβ binds to RAGE (the receptor for advanced glycation end -products) on the cell surface, thereby triggering reactions that could generate cytotoxic oxidizing stimuli (Yan et al., 1996). Blocking access of Aβ to the cell surface binding site(s) might retard progression of neuronal damage in AD. To date there are no specific pharmacological agents for blocking Aβ-induced neurotoxicity.

SUMMARY OF THE INVENTION

The invention relates to methods of treating a subject having Alzheimer's Disease or another disease or disorder characterised by amyloid β deposition, comprising the step of administering an antibody molecule which is targeted to β amyloid peptide or to fragment thereof. In another embodiment, the invention relates to an antibody molecule, which is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide and to a pharmaceutical composition comprising the same. In another embodiment, the invention relates to an antibody molecule, which is targeted to the free C or N-terminus of N-and/or C-terminus truncated amyloid β peptide fragment and to a pharmaceutical composition comprising the same.

In one embodiment, the invention relates to use of an antibody which is targeted to amyloid β peptide, or to fragment thereof for the manufacturing of a medicament for treating a subject having Alzheimer's Disease.

In one embodiment, the invention relates to use an antibody which is targeted to amyloid β peptide, or to fragment for manufacturing a medicament for treating a subject having a disease or disorder characterized by amyloid β deposition. Examples of such disorders include: mild cognitive impairment (MCI), cerebral

amyloid angiopathy or congiophylic angiopathy, Down Syndrome-associated Alzheimer's disease, and inclusion-body myositis.

In another embodiment, the invention relates to use of an antibody which is targeted to an amyloid β peptide, or to fragment thereof, for maufacturing a medicament for delaying or inhibiting or suppressing accumulation of amyloid β peptide or fragment thereof in the brain.

In another embodiment, the invention relates to use of an antibody which is targeted to amyloid β peptide, or fragment thereof for manufacturing a medicament for delaying or inhibiting or suppressing the neurotoxicity of amyloid β peptide or fragment thereof.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide, wherein the first amino acid of the free N-terminus of amyloid β-peptide is aspartate

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free N terminus of N- and/or C-terminus-truncated amyloid β peptide fragment.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-39, Aβ1-40, Aβ1-41, or Aβ1-43.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free C-terminus of N- and/or C-terminus-truncated amyloid β peptide fragment.

In another embodiment, the invention relates to a single chain antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-42.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of the amyloid precursor protein (APP) and the products of a, β, and γ -secretase cleavage. The general locations of various domains are indicated along with the cleavage sites (α, β, γ) for secretases

Figure 2 shows the amino acid sequence (SEQ ID N0:1) of the region in βAPP from which β -amyloid peptides (A β) are derived. The arrows indicate the α-, β - or γ - secretase cleavage sites. The amino acid residues corresponding to the synthetic peptides that can be used as immunogens are indicated underneath the sequence by line segments.

Figures 3A-3D schematically show the structure of a whole antibody (Fig. 3A) with the variable domain of heavy (V_H) and light (V_L,) chains and the constant domain(s) of light (C_L) and heavy (C_H1 C_H2, C_H3) chains, a Fab fragment (Fig. 3B), a Fv fragment (Fig. 3C), and a single chain Fv fragment (scFv) (Fig. 3D). The Fab fragment shown in Fig. 3B consists of a variable domain of heavy V_H and light V_L chain and the first constant domain (C_H1 and C_L) joined by a disulfide bridge. The Fv fragment shown in Fig. 3C represents the antigen binding portion of an antibody formed by a non- covalently linked variable region complex (V_H-V_L), whereas the single chain Fv shown in Fig. 3D joins the variable heavy V_H, with the variable light V_L, chain via a peptide linker.

Figure 4 A-C: Figure 4A shows a schematic representation of the amyloid precursor protein (APP) and its functional domain; Figure 4B is an enlargement of the Aβ peptide and its N-terminal sequences, showing the positions from which Peptides A and C were derived. Figure 4C is the one letter amino acid code for the seqeunce of APP and the experimental peptides A and C.

Figure 5 shows the generation of *epitope-specific* antibodies. Specific binding of Sample 1 to the immungen (Peptide A) in a standard ELISA assay is inhibited by the same peptide, by the $A\beta_{1-40}$ Peptide and by Peptide C, which spans the cleavage site of Aβ within the precursor molecule APP.

Figure 6 shows the generation of *free end-epitope-specific* antibodies. Specific binding of Sample 2 to the immungen (Peptide A) in a standard ELISA assay is inhibited by the same peptide, by the $A\beta_{1-40}$ Peptide but NOT by Peptide C, which spans the cleavage site of Aβ within the precursor molecule APP.

Figures 7 and 8 show the generation of *free end-epitope-specific* hybridomas. Clones 4D12 and 2A10 are two examples of hybridomas, which recognize the positive control immunizing peptide much greater than they recognize the negative control spanning peptide.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, the invention relates to a method of treating a subject having Alzheimer's Disease, comprising the step of administering an antibody which is targeted to amyloid β peptide, or to fragment thereof thereby treating the subject having Alzheimer's Disease.

In another embodiment, the invention relates to a method of treating a subject having a disease or disorder, characterized by amyloid beta deposition comprising the step of administering an antibody which is targeted to amyloid β peptide, or to fragment thereof, thereby treating the subject having a disease or disorder, characterized by amyloid beta deposition.

The other disease or disorder characterized by amyloid beta deposition are, for example without being limited, mild cognitive impairment (MCI), cerebral amyloid angiopathy or congiophylic angiopathy, Alzheimer's disease associated with Down Syndrome, and inclusion-body myositis.

The terms "amyloid beta", or "Aβ", or "amyloid β", or "beta amyloid" are all refered to interchangeably hereinabove to any of the amyloid β species. Such proteins are typically of about 4 kDa. Several different amino-termini and heterogeneous carboxyl-termini sequences have been observed based on characterization of the peptide amyloid β from Alzheimer's disease tissue and from cultured cells (Glenner and Wong (1984) Biochem Biophys Res Commun 120:885-890; Joachim et al. (1988) Brain Res 474:100-111; Prelli et al. (1988) J Neurochem

51:648-651; Mori et al. (1992) J Biol Chem 267:17082-17806; Seubert et al. (1992) Nature 359:325-327; Naslund et al. (1994) Proc Natl Acad Sci USA 91:8378-8382; Roher et al. (1993) Proc Natl Acad Sci USA 90:10836-10840; Busciglio et al. (1993) Proc Natl Acad Sci USA 90:2092-2096; Haass et al. (1992) Nature 359:322-325). Specifically, with regard to the carboxyl-termini, the amyloid β peptide has been shown to end at position 39, 40, 41, 42, 43, or 44 where position 1 is the aspartate of the amyloid β sequence as defined by Glenner and Wong (1984) Biochem Biophys Res Commun 120:885-890.

While recognizing the dominant role of full-length Aβ peptides, the present invention is not limited solely to these forms. Thus, notwithstanding the importance of full-length Aβ peptides as major therapeutic targets, the invention also envisages using antibodies that are free end-specific for other Aβ peptide-derived fragments that are neurotoxic and/or can form fibrillar deposits. Importantly, free end-specific antibodies that recognize N- or C-terminus truncated Aβ-peptides species should not react with the amyloid precursor protein from which they were derived.

The terms "amyloid β fragment" or "heterogeneous amyloid β" or "truncated amyloid β" interchangeably refer to fragments derived from the full length beta amyloid peptide defined above. Biochemical studies have demonstrated that in addition to an L-aspartate at positions 1, Aβ peptides can begin with a raceminzed or isomerized aspartate. Prominent N-terminus truncated Aβ isoforms begin with a cyclized glutamate (pyroglutamate) residue at position 3, pyroglutamate at position 11, and leucine at position 17 (Geddes et al 1999). Support for the fact that these isoforms contribute to the pathogenesis of Alzheimer's Disease is also based on studies which demonstrate 1) N-terminus truncated forms aggregate more readily and are more toxic in vitro than Aβ1-40 or Aβ1-42 (Pike et al. 1995) and 2) N-terminus truncated forms are among the earliest isoforms detected in plaques and may form a nidus for plaque formation (Theo et al 1995). Aβ17-42 (the p3 peptide) for example, is prevalent in AD brains but absent or sparse in aged, non-AD brains (Higgins et al. 1996). Studies of AD amyloid with high-resolution reverse-phase liquid chromatography and mass spectrometry confirm that additional N-terminus

truncated forms are invariably present, including Aβn-42 (n=1-11) and Aβ3-40 (Larner 1999). Studies of Aβ secreted into media of various cultured cells and cell lines transfected with differing APP constructs have identified Aβ species beginning at positions 2, 3, 4, 5, 6, 9, 11, 16, 17, 18, 19, 20, 24 (Busciglio et al 1993, Haas et al 1992, Haas et al 1994). The "nonamyloidogenic" p3 fragment (amyloid beta 17-42) is a major constituent of Down's syndrome cerebellar preamyloid (Lalowski M et al., J Biol Chem 1996 Dec 27;271(52):33623-31). Removal of this major forms, or limiting its neurotoxicity, can therefore be expected to slow progression of Down syndrome-associated Alzheimer's Disease and delay onset in susceptible individuals. Thus, the antibody of the present invention is directed in one embodiment to the N or to the C terminus of an amyloid beta peptide and in one embodiment is directed to the N or to the C- terminus of any fragment derived from the amyloid beta peptide.

By "antibody" is meant an immunoglobulin protein, which is capable of binding an antigen. Antibody as used herein is meant to include the entire antibody as well as any antibody fragments (e.g. $F(ab')_2$, Fab', Fab, Fv) capable of binding the epitope, antigen or antigenic fragment of interest. In one embodiment the antibody of the invention is a monoclonal antibody, a humanized antibody, a chimeric antibody, a bispecific antibody a scFv antibody or a F(ab) or fragments thereof.

The term "monoclonal antibody" is referred hereinabove to an immunological effective fragment as well as single chain forms.

The term "humanized antibody" is referred herein above to an antibody in which the complementary-determining regions (CDRs) of a mouse or other non-human antibody are grafted onto a human antibody framework. By human antibody framework is meant the entire human antibody excluding the CDRs.

The term "chimeric antibody" refers to an antibody in which the whole of the variable regions of a mouse or rat antibody are expressed along with human constant regions.

The term "artificial antibody" referes hereinabove to antibodies made by using molecular imprinting techniques as described in US Pat No. 5,630.978. Briefly, the

method for the preparing of the artificial antibody is as follows: (i) polymerization of functional monomers around the biologically active molecule, which is the antibody to amyloid β peptide or fragment thereof (the template); (ii) removal of the template molecule; and then (iii) polymerization of a second class of monomers in the void left by the template, to provide a new organic molecule which exhibits one or more desired properties which are similar to that of the template. Thus, in one embodiment, the invention relates to artificial antibodies which are molecularly imprinted polymers (MIPs) that selectively recognize amyloid β peptides and fragments thereof and act as antibodies and to their use in treating Alzheimer's Disease and other diseases which are characterized by amyloid β deposition.

The term "treating" is referred hereinabove to delay or prevent the onset slow the progression or ameliorate the symptoms related to Alzheimer's Disease or other disease or disorder characterized by amyloid β deposition.

In another embodiment, the invention relates to a method for delaying or inhibiting or suppressing the accumulation of an amyloid β peptide or fragment thereof, comprising the step of administering an antibody which is targeted to an amyloid β peptide, or to fragment thereof, thereby delaying or inhibiting or suppressing accumulation of amyloid β peptide or fragment thereof in the brain.

In another embodiment, the invention relates to a method for delaying or inhibiting or suppressing the neurotoxicity of amyloid β peptide or fragment thereof, comprising the step of administering an antibody which is targeted to amyloid β peptide, or fragment thereof, thereby delaying or inhibiting or suppressing the neurotoxicity of amyloid β peptide or fragment thereof.

Thus, the invention relates to the use of antibodies to amyloid β peptides as a method to selectively inhibit accumulation and/or neutralize the cytotoxicity associated with amyloid β species. The most effective target for end-specific antibodies as therapy for Alzheimer's Disease is likely to be Aβ1-40 which forms the bulk of circulating amyloid β peptide (human CSF, plasma, and urine), or the more toxic but less abundant Aβ1-42 and Aβ1-43 species that can seed amyloid deposition. Neuritic plaques and vascular amyloid deposits contain an abundance of these forms and the consensus that has evolved from genetic and biochemical

analyses of human tissue and transgenic mouse models is that full-length forms of amyloid β peptide are the key players in the pathogenesis of Alzheimer's Disease.

The antibody or the pharmaceutical composition which contain the same, will be administered to the periphery, or a pharmaceutical composition containing an antigen to elicit such antibodies, or in another embodiment, the antibody of the invention can be added directly to the brain by intracranial or intracranially injection.

In one embodiment, the antibody of the invention will cross the blood brain barrier and will form a complex with the amyloid β protein or fragment in the brain.

In one embodiment, the invention relates to methods of treating central nervous system (CNS) conditions by peripheral administration of antibodies. The usefulness of IV Ig treatment in epilepsy was assessed by Engelen BG et al.(J. Neurol Neurosurg Psychiatry 1994 Nov 57 Supp 21-5). The conclusion of these authors from the study on cerebrospinal fluid IgG concentrations before and after IV Ig treatment in patients with epilepsy was that the main component of IV Ig preparation crosses the blood CSF barrier and significantly increases CSF IgG concentration, and may reach the brain and act centrally.

The presence of IgG in the central nervous system was demonstrated by immunocytochemistry and showed a close anatomical relationship between the distribution of this protein and the blood-brain barrier. IgG was immunolocalized in the normal rat brain by using monoclonal and polyclonal antibodies to IgG and its subclasses.

The passage of intravenous immunogloubulin and interactions with the CNS was summarized in a review by Wurster et al. (J. Neurol Neurosurg Psychiatry 1994 Nov 57 Supp 21-5).

In one embodiment, the antibody of the invention penetrates into the brain cells through the blood brain barrier (BBB) by using methods or carriers, which details are provided below.

Preferred compounds to be added to formulations to enhance the solubility of the antibodies are cyclodextrin derivatives, preferably

hydroxypropyl-gamma-cyclodextrin. Drug delivery vehicles containing a cyclodextrin derivative for delivery of peptides to the central nervous system are described in Bodor, N., et al. (1992) Science 257:1698-1700.

Accordingly, use of an antibody of the invention in combination with a cyclodextrin derivative may result in greater inhibition of β amyloid neurotoxicity than use of the modulator alone. Chemical modifications of cyclodextrins are known in the art (Hanessian, S., et al. (1995) J. Org. Chem. 60:4786-4797). In addition to use as an additive in a pharmaceutical composition containing a modulator of the invention, cyclodextrin derivatives may also be useful as modifying groups and, accordingly, may also be covalently coupled to free end specific β amyloid antibody to form a modulator compound of the invention.

Low et al., U.S. Patent 5,108,921, reviews available methods for transmembrane delivery of molecules such as proteins and nucleic acids by the-mechanism of receptor mediated endocytotic activity. These receptor systems include those recognizing galactose, mannose, mannose-6-phosphate, transferrin, asialoglycoprotein, transcobalamin (vitamin B_{12}), α-2 macroglobulins, insulin and other peptide growth factors such epidermal growth factor (EGF). Low et al. also teaches that nutrient receptors, such as receptors for biotin and folate, can be advantageously used to enhance transport across the cell membrane due to the location and multiplicity of biotin and folate receptors on the membrane surfaces of most cells, and the associated receptor mediated transmembrane transport processes. Thus, a complex formed between a compound to be delivered into the cytoplasm and a ligand, such as biotin or folate, is contacted with a cell membrane bearing biotin or folate receptors to initiate the receptor mediated trans-membrane transport mechanism and thereby permit entry of the desired compound into the cell.

A biotin ligand can be attached to a DM molecule, for example, by incorporating commercially available biotinylated deoxynucleotide triphosphates, e.g., biotin-14-MTP or biotin-14-dCTP from Invitrogen Life Technologies, Carlsbad, CA, using terminal deoxynucleotidyl transferase (Karger, B.D., 1989). Biotin-14-dATP is a MTP analog with biotin attached at the 6-position of the purine base by a 14- atom

linker and biotin-14-dCTP is a dCTP analog with biotin attached at the N^4 -position of the pyrimidine base also by a 14-atom linker.

In one embodiment, the antibody of the invention can be delivered by liposomes, which is well covered in the scientific literature and in patent publications.

In another approach for enhancing transport across the BBB, a peptidic or peptidomimetic modulator is conjugated to a second peptide or protein, thereby forming a chimeric protein, wherein the second peptide or protein undergoes absorptive-mediated or receptor-mediated transcytosis through the BBB. Accordingly, by coupling the modulator to this second peptide or protein, the chimeric protein is transported across the BBB. The second peptide or protein can be a ligand for a brain capillary endothelial cell receptor ligand. For example, a preferred ligand is a monoclonal antibody that specifically binds to the transferrin receptor on brain capillary endothelial cells (see e.g., U.S. Pat. Nos. 5,182,107 and 5,154,924 and PCT Publications WO 93/10819 and WO 95/02421, all by Friden et al.). Other suitable peptides or proteins that can mediate transport across the BBB include histones (see e.g., U.S. Pat. No. 4,902,505 by Pardridge and Schimmel) and ligands such as biotin, folate, niacin, pantothenic acid, riboflavin, thiamin, pryridoxal and ascorbic acid (see e.g., U.S. Pat. Nos. 5,416,016 and 5,108,921, both by Heinstein). Additionally, the glucose transporter GLUT-1 has been reported to transport glycopeptides (L-serinyl-β-D-glucoside analogues of [Met5]enkephalin) across the BBB (Polt, R. et al. (1994) Proc. Natl. Acad. Sci. USA 91:7114-1778). Accordingly, a modulator compound can be coupled to such a glycopeptide to target the modulator to the GLUT-1 glucose transporter. For example, a modulator compound which is modified at its amino terminus with the modifying group Aic (3-(O-aminoethyl-iso)-cholyl, a derivative of cholic acid having a free amino group) can be coupled to a glycopeptide through the amino group of Aic by standard methods. Chimeric proteins can be formed by recombinant DNA methods (e.g., by formation of a chimeric gene encoding a fusion protein) or by chemical crosslinking of the modulator to the second peptide or protein to form a chimeric protein. Numerous chemical crosslinking agents are known (e.g., commercially available from Pierce, Rockford Ill.). A crosslinking agent can be chosen which allows for

high yield coupling of the modulator to the second peptide or protein and for subsequent cleavage of the linker to release bioactive modulator. For example, a biotin-avidin-based linker system may be used.

In yet another embodiment for enhancing transport across the BBB, the modulator is encapsulated in a carrier vector, which mediates transport across the BBB. For example, the modulator can be encapsulated in a liposome, such as a positively charged unilamellar liposome (see e.g., PCT Publications WO 88/07851 and WO 88/07852, both by Faden) or in polymeric microspheres (see e.g., U.S. Pat. No. 5,413,797 by Khan et al., U.S. Pat. No. 5,271,961 by Mathiowitz et al. and 5,019,400 by Gombotz et al.). Moreover, the carrier vector can be modified to target it for transport across the BBB. For example, the carrier vector (e.g., liposome) can be covalently modified with a molecule which is actively transported across the BBB or with a ligand for brain endothelial cell receptors, such as a monoclonal antibody that specifically binds to transferrin receptors (see e.g., PCT Publications WO 91/04014 by Collins et al. and WO 94/02178 by Greig et al.).

An antibody of the invention can be formulated into a pharmaceutical composition wherein the antibody is the only active compound or, alternatively, the pharmaceutical composition can contain additional active compounds. For example, two or more antibody compounds may be used in combination. Moreover, an antibody of the invention can be combined with one or more other agents that have anti-amyloidogenic properties. For example, an antibody can be combined with the non-specific cholinesterase inhibitor tacrine (COGNEX.RTM., Parke-Davis).

Other methods that may be developed from time to time are also contemplated.

Once delivered into the brain the specific antibody molecules will transfer into the extracellular space, interstitial fluid and cerebrospinal fluid. The specific antibody molecules then form a soluble complex with Aβ peptide. These soluble specific-Aβ peptide-antibody complexes reduce, in one embodiment, the depostion of Aβ peptides into amyloid plaques and attenuate Aβ peptide -induced neurotoxicity by clearing Aβ peptides from the central nervous system through drainage of the extracellular space, interstitial fluid and cerebrospinal fluid into the general blood circulation where they will be eliminated by protease digestion. Accordingly, the

accumulation of newly secreted soluble Aβ peptides responsible for amyloid deposition and Aβ-induced neurotoxicity is inhibited.

Furthermore, clearance of soluble amyloid - β peptides in accordance with the present invention is expected to reduce the inflammatory process observed in Alzheimer's Disease by inhibiting, for example, amyloid -β-induced complement activation and cytokine release, and blocking also the interaction of Aβ with cell surface receptors such as the RAGE receptor.

In another embodiment, once the antibodies of the invention bind to the amyloid β peptide they can elicit a cellular immune response (i.e. via activation of the Fc receptor). Fc receptor can distinguish between an antibody, which is bound to an antigen, and a free antibody. The result will be that the Fc receptors will enable accessory cells, which are usually not capable of identifying target antigens to target and engulf amyloid β peptide. In this scenario, the requirement for a stoichiometric relationship between antibody and antigen may be eliminated. As a consequence, less free-end specific antibody will be required to penetrate the BBB for elimination of deposited amyloid β peptides.

In another embodiment, the interaction of Aβ with APOE4 gene product will be reduced by the adding of the antibody of the invention.

In one embodiment, the antibody or the pharmaceutical composition which contain the same, will block peripheral Aβ peptide from entering the CNS, thus reducing accumulation of amyloid plaques in the brain.

In another embodiment, the pharmaceutical composition and the antibodies of the present invention will delay the onset and inhibit or suppress the progression of Alzheimer's Disease by having a peripheral effect. The clearance or the removal of the amyloid beta from the periphery will change the equilibrium of the amyloid beta in the blood and as a result in the brain. Recent studies have shown that amyloid beta is transported from the cerebrospinal fluid to the plasma with an elimination half-life from brain of about half an hour. Thus, the antibody can affect the amyloid beta level in the plasma, cause accumulation of central amyloid beta in the plasma and as a result reduce the amyloid β deposition in the brain.

In one embodiment, the invention provides an antibody molecule which is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide and/or fragments thereof which is capable of discrimination between an Aβ peptide and the amyloid protein precursor. The term "free end specific" means a molecule which binds specifically to a free terminus/end of an Aβ peptide or to any fragment thereof to slow down or prevent the accumulation of amyloid - β peptides in the extracellular space, interstitial fluid and cerebrospinal fluid and to block the interaction of Aβ peptides with other molecules that contribute to the neurotoxocity of Aβ.

Without being limited, the method of detecting provides an antibody molecule which is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide and/or fragments thereof namely, the 'spanning peptide ELISA detection method' is as follows: antibodies that bind sequences in the N-terminus region of amyloid β peptide, (for example residues 1-5 of Peptide A or Asp-Ala-Glu-Phe-Arg; see Figure 4), and also bind the identical sequence of amino acids in the spanning peptide C, which corresponds to sequences of the amyloid precursor protein (APP), are eliminated from the screen. Only antibodies that bind (NH_2)Asp-Ala-Glu-Phe-Arg with a free N-terminus (for example, the heptamer peptide or full length Aβ1-40), but do not bind the spanning peptide, are selected in this detection system. These selected antibodies should be described as 'free N-end-specific' because their binding epitope incorporates both a high affinity recognition element for a free amino (NH_2) group, in addition to recognition of the N-terminal amino acid residues of the particular amyloid-β peptide. A similar detection system is envisaged for selecting antibodies that are C-end-specific using a spanning peptide that corresponds to a contiguous sequence of amino acids on both sides of the C-terminal cleavage sites in APP. This exquisite sensitivity of free-end specific antibodies is required so as not to affect the normal biological functions of the transmembrane receptor-like APP molecule that is implicated in several important physiological roles (such as mediation in adhesion, growth promoting effects, neuroprotection, neuritic outgrowth, recycling of synaptic vesicles, regulation of apoptosis inhibition of serine proteases, receptor and signal transduction functions,

calcium metabolism and nucleic acid transcription). Thus, in one embodiment, the invention utilizes *free-end specific* antibodies to inhibit the accumulation of amyloid β peptides, to ameliorate or prevent the neurotoxic consequences of amyloid deposition, to slow Alzheimer's Disease or other diseases characterized by amyloid β deposition progression or to delay their onset.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide. In another embodiment the invention relates to an antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide, wherein the first amino acid of the free N-terminus of amyloid β-peptide is aspartate.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free N terminus of N- and/or C-terminus-truncated amyloid β peptide fragment.

In one embodiment, the free end specific antibody is specific to amino acids 1-3 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group. In one embodiment, the free end specific antibody is specific to amino acids 1-4 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group. In one embodiment, the free end specific antibody is specific to amino acids 1-5 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group. In one embodiment, the free end specific antibody is specific to amino acids 1-6 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group. In one embodiment, the free end specific antibody is specific to amino acids 1-7 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group. In one embodiment, the free end specific antibody is specific to amino acids 1-8 of the N-terminus-truncated amyloid β-peptide in addition to a free amino (NH_2) group.

As shown in Examples, the 'spanning peptide ELISA detection method' may also be applied to the free N-terminus of any one of the N-terminus-truncated amyloid β-peptides species that are found to be important in the pathogenesis of Alzheimer's disease (Masters CL et al 1985, Haass et al 1993, Miller DL et al 1993, Vigo-Pelfrey C et al 1993, Naslund et al 1994, Theo TC et al 1995). The present specification

describes in one embodiment the use of short peptides such as SEQ ID NO:2 (Asp-Ala-Glu-Phe-Arg-Cys) and SEQ ID NO:3 (Asp-Ala-Glu-Phe-Arg-His-Asp-Cys that correspond to the amino acid sequence at the N-terminus of amyloid β peptide starting at position 1 (Asp) as examples of immunogens for the production of free end- specific antibodies. When using these immunogens, in one embodiment the lack of reactivity with a spanning peptide (SEQ ID NO:7 Glu-Ile-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-His) in the 'spanning peptide ELISA detection method' is tested. Similarly, immunization or selection with sequences corresponding to N-terminus-truncated amyloid β-peptides species such as Aβ11-40/42, followed by screening with the appropriate spanning peptide could also be employed in this invention. Examples of such sequences are included herein: SEQ ID NO:9 Glu-Val-His-His-Gln-Cys, corresponding to residues 11-15 of the full length Aβ peptide, with an additional cysteine residue for conjugation purposes is an example of an immunizing peptide; SEQ ID NO:10 Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln is an example of a spanning peptide which would be used in the 'spanning peptide ELISA detection method' in order to isolate end-specific antibodies.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-39, Aβ1-40, Aβ1-41, or Aβ1-43.

In another embodiment, the invention relates to a single chain antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-42.

In another embodiment, there is provided an antibody which is free-end specific for the free C-terminus of the amyloid β-peptide Aβ1-40, which antibody does not bind to the amyloid β-precursor protein from which the amyloid β-peptide is proteolytically derived.

In another embodiment, there is provided an antibody free-end specific for the free C-terminus of the amyloid β-peptide Aβ1-43 which antibody does not bind to the amyloid precursor protein from which the amyloid β-peptide is proteolytically derived.

In another embodiment, the invention relates to an antibody that is free-end specific and is targeted to the free C-terminus of N- and/or C-terminus-truncated amyloid β peptide fragment. Thus, the invention also addresses heterogeneity at the C-terminus

where the longer forms, namely, Aβx-42/3, are more prone to aggregate as fibrils and can lead to aggregation of the more abundant Aβ1-40. For example without being limited, the invention envisages the therapeutic use of antibodies that are free end-specific for the C-terminus of each of the forms Aβx-39, Aβx-40, Aβx-41, Aβx-42, and Aβx-43. For example, in one embodiment, Aβ 42 has the following sequence:

H_2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH.

Aβ41, Aβ40 and Aβ39 differ from Aβ42 by the omission of Ala, Ala- Ile and Ala-Ile-Val respectively from the C-terminal end. Aβ43 differs from Aβ42 by the presence of a threonine at the C-terminus.

As examples, in one embodiment the use of certain antigenic sequences corresponding to short regions at the C-terminus of amyloid β peptide are described by SEQ ID No:4 Cys-Leu-Met-Val-Gly-Gly-Val-Val (Aβx-40), and SEQ ID No: 11 Cys-Gly-Gly-Val-Val-Ile-Ala-Thr (Aβx-43). Spanning peptides which may be used to detect C-terminal end-specific antibodies in the 'spanning peptide ELISA detection method' are SEQ ID NO: 12 (Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val) and SEQ ID NO: 13 (Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr), for Aβx-40 and Aβx-43, respectively. Analogous peptides can be designed for targeting Aβx-42 species. Thus, for example, without being limited, in a representative example using amyloid β 1-40, in one embodiment, the free end specific antibody is targeted to amino acids 33-40 of the C-terminus-truncated amyloid β-peptide in addition to a free carboxylic (COOH) group. In one embodiment, the free end specific antibody is targeted to amino acids 34-40 of the C-terminus-truncated amyloid β-peptide in addition to a free carboxylic (COOH) group. In one embodiment, the free end specific antibody corresponds to amino acids 36-40 of the C-terminus-truncated amyloid β-peptide in addition to a free carboxylic (COOH) group. In one embodiment, the free end specific antibody corresponds to amino acids 37-40 of the C-terminus-truncated amyloid β-peptide in addition to with a free carboxylic (COOH) group. Please note that the amyloid β-

peptide 1-40 served as a representative example; the same fragments can be derived from amyloid β-peptide 1-39, 1-41, 1-42 and 1-43.

As shown in Fig. 1 (see Schehr, 1994), and discussed in the Background Art section, the amyloid protein precursor (APP) is believed also to serve as a precursor for a proteolytic product, soluble -amyloid protein precursor (sAPP), thought to have growth promoting and neuroprotective functions. It will be readily appreciated by those of skill in the art that the introduction/administration of free end specific molecules will not interfere with the normal biological functions of APP or sAPP that are not associated with the formation of Aβ peptides.

Single-chain antibodies as free end specific molecules can also be produced according to the present invention. These single chain antibodies can be single chain composite polypeptides having free end -specific Aβ peptide binding capability and comprising a pair of amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L, or single chain Fv). Both V_H and V_L may copy natural antibody sequences, or one or both of the chains may comprise a CDR construct of the type described in U.S. Patent 5,091,513. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a peptide linker. Methods of production of such single chain antibodies, e.g., single Fv (scFv), particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are characterized or can be readily ascertained by sequence analysis, may be accomplished in accordance with the methods described, for example, in U.S. Patent 4,946,778, U.S. Patent 5,091,513, U.S. Patent 5,096,815, Biocca et al., 1993, Duan et al., 1994, Mhashilkar et al., 1995, Marasco et al., 1993, and Richardson et al., 1995. Figures 3A-3D (from Biocca et al., 1995) schematically show an intact antibody (Fig. 3A), a Fab fragment (Fig. 3B), a Fv fragment consisting of a non- covalently linked variable region complex (V,-V, (Fig. 3C) and a single chain Fv antibody (Fig. 3D).

The design of immunogenic peptides for use in immunization in an animal and the generation of antibody producing hybridomas is based on similar peptides that have been previously used by several laboratories to generate polyclonal antibodies for in vitro use. that recognize the free termini of Aβ species (Harrington et al., 1993;

Iwatsubo et al., 1994; Konig et al., 1996; Murphy et al., 1994; Gravina et al., 1995). While peptides of longer lengths have in some instances been used successfully to generate Aβ end-specific antibodies, Theo and co-workers (1993; 1994) established that there is a length of five amino acids for any given peptide which ensures that the specific free amino group at the N-terminus constitutes an essential part of the epitope recognized by the new antibody. Thus, an antibody generated against an immunogenic peptide is evaluated for the selectivity of the antibody in its recognition of a free N- or C-terminus of an Aβ peptide. A competitive inhibition assay, using Enzyme-Linked Immunosorbant Assay (ELISA) or immunoprecipitation with peptides corresponding to different regions of Aβ and the region immediately preceding the β - secretase cleavage site in the extracellular domain of βAPP, can determine the selectivity of the antibody.

Those of skill in the art will appreciate that a cysteine residue can be added to the end of the above immunogenic peptides opposite from the end corresponding to the free N-terminus or the free C-terminus of Aβ peptides to facilitate coupling to a carrier protein. Keyhole limpet hemocyanin (KLH), ovalbumin and bovine serum albumin (BSA) are non-limiting examples of proteins that can be used as carriers for immunogens. The presence of an N-terminal or C- terminal cysteine residue on the synthetic immunogen peptides provides a free sulfhydryl group for covalent coupling to a maleimide-activated protein. A heterobifunctional reagent, such as an N-maleimido-6-aminocaproyl ester or a m- maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), is used to covalently couple the synthetic immunogenic peptide to the carrier protein (see for example, Hartlow, E. et al., Antibodies : A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1988). Commercial kits are also readily available for use in coupling peptide antigens to maleimide-activated large carrier proteins.

The invention further provides a hybridoma cell producing monoclonal antibody or a single chain antibody that is free end-specific for the N-terminus or the C-terminus of an amyloid β peptide or fragment thereof and discriminates between an Aβ peptide and the amyloid protein precursor from which it is proteolytically derived. The hybridomas producing the monoclonal antibodies of the present invention are

produced following the general procedures described by Kohler and Milstein, Nature, 256, p. 495 (1975). In that procedure, hybridomas are prepared by fusing antibody-producing cells (typically spleen cells of mice previously immunized with an amyloid beta as antigen source) to cells from an immortal tumor cell line using somatic cell hybridization procedures.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humanized mice, humans, and others may be immunized by injection with the relevant epitope or with any fragment or oligopeptide thereof, which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol.

The hybridomas resulting from the fusion process are allowed to grow. Thereafter, the resulting supernatants are screened using immunoassay procedures to detect antibodies present in the supernatants capable of binding to the specific antigens.

In another embodiment, combinatorial antibody library technology, i.e., antigen based selection from antibody libraries expressed on the surface of M13 filamentous phage, can be used for the generation of monoclonal antibodies and possesses a number of advantages relative to hybridoma methodologies (Huse, et al, 1989: Barbas, et al. 1991; Clackson, et al, 1991: Burton and Barbas, 1994). The antibody of the invention may be generated from phage antibody libraries. The general methodologies involved in creating large combinatorial libraries using phage display technology is described and disclosed in U.S. Pat. No. 5,223,409 issued Jun. 29, 1993.

Once monoclonal antibodies are generated, the selectivity and binding affinity (Kd) can be evaluated by ELISA, and in vitro bioassays can be performed on the antibodies to test for the efficacy of the Aβ end -specific antibodies in blocking Aβ - induced cytotoxicity. In vitro bioassays can also be performed on the antibodies to test for the lack of interference with function of the amyloid precursor protein APP.

In another embodiment, the antibodies will be produced in vivo, in the subject in need, by administering of an antigen such as amyloid β peptide or fragments thereof.

The titer of the antibodies will be determined by techniques which are known to one skilled in the art and additional antigen will be adminstered if required.

In another embodiment, there is provided a pharmaceutical composition comprised of the antibodies described above and a method of using the composition for the inhibition of the accumulation of amyloid β peptides in the extracellular milieu of a neuron. The diminished accumulation of amyloid β peptides will further delay the progression of the Alzheimer's Disease or other diseases characterized by amyloid beta deposition, in a subject in need.

In one embodiment, the composition includes an antibody in a therapeutically or prophylactically effective amount sufficient to inhibit the neurotoxicity of natural β amyloid peptides, and a pharmaceutically acceptable carrier. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as slowed progerssion of Alzheimer's Disease, delayed onset, reduction or reversal of amyloid β deposition and/or reduction or reversal of Aβ neurotoxicity. A therapeutically effective amount of the antibody of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the modulator to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modulator are outweighed by the therapeutically beneficial effects.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting the rate of amyloid deposition and/or amyloid β neurotoxicity in a subject predisposed to amyloid β deposition. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

One factor that may be considered when determining a therapeutically or prophylactically effective amount of an antibody to amyloid β is the concentration of natural amyloid β in a biological compartment of a subject, such as in the

cerebrospinal fluid (CSF) or the plasma of the subject. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens could be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

The pharmaceutical composition can be administered subcutaneously, intravenously, intradermally, intramuscularly, intraperitoneally, intracerebrally, intranasally, orally, transdermally, buccally, intra-arterially, intracranially, or intracephalically. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Preferably, the carrier can be suitable for intravenous, intraperitoneal or intramuscular administration. Alternatively, the carrier is suitable for administration into the central nervous system (e.g., intraspinally or intracerebrally). In another embodiment, the carrier is suitable for oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances

is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the antibody can be administered in a time-release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., antibody to amyloid β in the required amount) in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of

preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Topical application can result from intransdermal or intradermal application. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof. Alternatively, transdermal delivery can be achieved using skin patch or using transferosomes.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the active compounds of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone; nonpolymer systems that are lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di and triglycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings, compressed tablets using conventional binders and excipients, partially fused implants and the like. In addition, a pump-based hardware delivery system can be used, some of which are adapted for implantation.

A long-term sustained release implant also may be used. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well known to those of ordinary skill in the art and include some of the release systems described above. Such implants can be particularly useful in treating conditions characterized by aggregates of amyloid β peptides by placing the implant near portions of the brain affected by such aggregates, thereby effecting localized, high doses of the compounds of the invention.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

EXAMPLES

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLE 1

Demonstration of the "spanning peptide" ELISA detection methodology in detecting antibodies that are free-end specific for amyloid β peptides

Peptide H_2N-Asp-Ala-Glu-Phe-Arg-aminohexanoate-Cys-amide was conjugated to BSA through a SMCC linker. Swiss Webster mice were immunized with 100 μg of this conjugate in Freund's complete adjuvant and then boosted twice with a further 100 μg of conjugate in Freund's incomplete adjuvant. Antisera were screened using the 'spanning peptide ELISA detection method', as described in the specification. Briefly, Aβ1-40 was coated onto 96-well plates, which were then incubated with samples in the presence of competing peptides A (immunogen), C (spanning peptide), or Aβ1-40 itself. The common result is shown in Figure 5. As expected, antibodies produced by these animals bind residues 1-5 of Aβ (peptide A), which was the peptide used for immunization, and the full-length Aβ1–40 peptide. However, these antibodies are also reactive with the Aβ1-5 epitope when flanked by additional sequences on its N-terminus (peptide C), as is the case in the intact amyloid precursor protein APP. Such antibodies may be referred to as epitope-specific (for sequences 1-5 of the N-terminus of Aβ).

A much more rare result seen upon testing of samples is shown in Figure 6. These antibodies bind the immunizing peptide (Aβ1-5) and the Aβ1-40 sequence, but do not recognize the peptide derived from the region which spans the same 5 amino acid sequence in the full length APP (peptide C). The exquisite selectivity of these

antibodies lies in their ability to bind the 1-5 sequence only when positioned at the free N-terminus of the molecule; these antibodies are referred to as *free end-specific*.

EXAMPLE 2

Demonstration of the "spanning peptide" ELISA detection methodology in selecting hybridomas that are free-end specific for amyloid β peptides

Peptide H_2N-Asp-Ala-Glu-Phe-Arg-His-aminohexanoate-Cys-amide was conjugated to KLH through an MBS linker. Balb/c x C57Bl/6 F1 mice were immunized with 50 μg of this conjugate in Freund's complete adjuvant and then boosted four times with a further 50 μg of conjugate in Freund's incomplete adjuvant. Antisera were tested and fusions performed using standard methods. Hybridomas were screened using the 'spanning peptide ELISA detection method', as described in the specification. Briefly, either positive peptide (H_2N-Asp-Ala-Glu-Phe-Arg-His conjugated to BSA) or negative control 'spanning' peptide (acetyl-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-His) were coated onto 96-well plates, which were then incubated with hybridoma supernatants. As seen in Figures 7 and 8, some hybridomas recognize both peptides in a similar manner (i.e. clones 5E2, 2A8, and 2F8) or with insignificant differences (i.e. clones 4H9, 1C12, and 3H3). However, some clones demonstrate free-end specificity in that they recognize the positive peptide used for immunization much greater than they recognize the negative control spanning peptide, which in fact contains the same sequence used for immunization. Examples are seen in clones 4D12 and 2A10 of this fusion.

EXAMPLE 3

Prophetic Experiments

In vitro bioassays to test efficacy of Aβ end -specific antibodies in blocking Aβ fibril formation and Aβ-induced cytotoxicity

Aβ -induced neurotoxicity

The receptor for advanced glycation end products (RAGE) mediates some of the neurotoxic effects of Aβ on neurons and microglia (Yan et al., 1996).

End -specific antibodies are tested for their ability to inhibit the receptor-mediated neurotoxicity by competitive inhibition. The antibodies are tested both with purified RAGE receptor preparations and by measuring their effect on Aβ -induced cellular oxidant stress.

The RAGE receptor is purified from a bovine lung extract dissolved in tris-buffered saline containing octyl-p- glucoside (1%) and phenylmethylsulfonylfluoride (2 nm) and applied to a heparin hyperD column (Biosepra). The column is eluted with a gradient of NaCl and fractions with maximal binding of ^{125}I-labeled Aβ are identified. The fractions are pooled and loaded onto hydroxyapatite ultragel (Biosepra) and eluted with increasing concentrations of phosphate.

Fractions with maximal binding of ^{125}I-labeled. Aβ are applied to preparative non-reduced polyacrylamide SDS gels (10%).

The RAGE receptor protein M_r 50,000 is identified by Coommassie Blue staining and the region in adjacent lanes are cut and eluted. Competitive inhibition by the end-specific antibodies to binding of ^{125}I-labeled A β (1-40/1-42) to the RAGE receptor is determined in a number of ways: (1) different amounts (0-150 μg) of purified protein are immobilized on microtiter wells and incubated with 100 nM ^{125}I-labeled A β (1-40/1-42); (2) different amounts (0-250 nM) of ^{125}I -labeled A β (1-40/1-42) are incubated in microtiter wells pre-coated with 5 μg purified RAGE receptor; and (3) different amounts (0-500 μg/ml) of A β (1-40/1-42) are immobilized on microtiter wells and incubated with 50 nM ^{125}I-labeled RAGE

receptor. In each assay, the amount of ligand binding to the well in the presence of different amounts of antibody is determined by counting the amount of radioactivity in the wells with a gamma-scintillation counter.

To evaluate the efficacy of the different end-specific A β antibodies as inhibitors of Aβ-induced cellular oxidant stress, cultured mouse brain microvascular endothelial cells (Breitner et al., 1994) are incubated with 0.25 μM A β in the presence of different amounts of the antibodies, and cellular oxidant stress is assessed by measuring the dose-dependent generation of thiobarbituric acid-reactive substances using the TBARS assay as previously described (Dennery et al., 1990; Yan et al., 1996). In a parallel assay system (developed by Khoury et al., 1996), the inhibitory effects of the antibodies are tested on Aβ-induced production of oxygen-reactive species in N9 mouse microglial cells. N9 cells (5×10^4 are incubated at 37^0C in the presence of different amounts of the antibodies in 50 μl PD-BSA (phosphate-buffered saline lacking divalent cation having 1 mg/ml BSA) containing 1 μM H_2DCF (2',7'-dichlorofluorescein diacetate), a dye that fluoresces upon oxidation (Wan et al., 1993) on multispot slides coated with Aβ peptides. At various time points, aliquots of the culture medium are taken and the fluorescence is measured in a fluorescence plate reader (Cytofluor II).

Effect on interactions with proteoglycans:

The vascular cell derived heparan sulfate proteoglycan, perlecan, has been identified in all amyloid deposits and is implicated in the earliest stages of inflammation-associated amyloid induction through high-affinity binding interactions with Aβ (Snow et al. 1989; 1995). Binding of perlecan to Aβ imparts secondary and tertiary amyloid structural features which suggest that molecules that interfere with the interaction may prevent or arrest amyloidogenesis.

Free end-specific Aβ antibodies made to peptides that correspond to the N-terminus of the peptide are evaluated for their ability to block the binding of perlecan to the perlecan binding site in the N-terminus region of Aβ (Snow et al., 1995). These evaluations are based on a solid-phase binding assay using perlecan isolated from cultured endothelial cells prepared from calf thoracic aortas as described in detail by

(Snow et al. 1995). Polyvinyl micro-titer wells are coated with 100 µl of nitrocellulose solution and allowed to dry. Wells are then coated overnight at room temp with unlabeled perlecan to give 0.28 ug of bound perlecan per well, and blocked overnight at room temp with 200 µl of 5% non-fat dried milk.

Various quantities of ^{125}I Aβ (7000 cpm/pM) diluted in 100 µl of TBS/0.05% Tween 20 (TBST) are added in triplicate to the wells and incubated for 2.5 h at room temp on an orbital shaker. At the end of the incubation period, free ^{125}I A β is removed with six washes of TBST. Bound ^{125}I is extracted in 100 µl 1N sodium hydroxide and "bound" versus "free" radioactivity is quantitated by liquid scintillation counting. Scatchard analysis is performed after incubating ^{125}I-A β in the presence of increasing amounts of antibody.

Effects on Aβ Fibril Formation:

Amyloid fibril formation by the kinetically soluble peptides, such as Aβ 1- 40, can be nucleated or "seeded" by peptides such as Aβ 1-42 that include the critical C-terminal residues 41(Ile) and 42(Ala). The ability of C-terminus or N-terminus end-specific Aβ antibodies to block seeding by Aβ 1-42 or to prevent aggregation of amyloid peptides is tested using standard aggregation assays (Wood et al., 1996). The Aβ 1-40 peptide is solubilized to 5 mg/ml in 1,1,1,3,3,3-hexafluoro-2- propanol. The peptide is concentrated to dryness and is resolubilized in phosphate-buffered saline (PBS), pH 7.4, to a final concentration of 230µm. A solution of Aβ 1-42 (20 µm) is stirred for 3 days and sonicated for 30 min to produce amyloid fibrils. Preaggregated Aβ 1-42 at 2nM concentration is added to the supersaturated pH 7.4 incubation to seed aggregation of Aβ 1-40. Aggregate formation in the absence and in the presence of each Aβ end -specific antibody is determined by monitoring the turbidity of samples prepared in microtiter wells using a microtiter plate reader at 405 nm. The reaction is also monitored by thioflavin-T fluorescence as described by Wood et al. (1996). The ability of free-end-specific antibodies to promote disaggregation of amyloid peptide fibrils is tested by testing the displacement of VI-labeled amyloid aggregated peptides from a collagen matrix containing non-aggregated peptides coated onto 96-well microtiter-plastic-coated plates. In addition,

the ability of free-end-specific-antibodies to protect neurons from Aβ-induced damage is assessed by the trypan blue exclusion method, intracellular calcium measurements, scanning and transmission electron microscopy and by confocal microscopy.

Animal models to establish the therapeutic potential of the Aβ antibodies

Animal models may be required to demonstrate the potential of antibody administration to slow down or prevent the development of AD-like pathology in the brain. Although AD is a uniquely human disease, a number of transgenic mice that overexpress human APP and PS1 have been developed. The correlative appearance of behavioral, biochemical, and pathological abnormalities reminiscent of Alzheimer's Disease in these transgenic mice provides the opportunity to explore the usefulness of agents to slow down or prevent the Aβ-induced pathophysiology of the disease.

The APP/PS1 double transgenic model ((Holcomb et al, Nat Med 1998 , 4: 97-100) has a particularly robust, reproducible phenotype which occurs very rapidly with first amyloid deposits and behavioral impairment detectable by 12 weeks of age. These factors combine to produce a model for rapid screening and evaluation of a drug candidate for Alzheimer's disease. Importantly, the phenotype of PS1/APP is consistent with several key features of human Alzheimer's disease and includes, for example, markers of oxidative stress, reactive gliosis, inflammation, neurodegeneration, abnormal neuronal growth, reorganization of cholinergic terminals, and the presence of hyperphosphorylated tau, an intermediate in tangle formation. Unlike many other models of cognitive impairment, PS1/APP has a selective "working memory" loss that is not accompanied by motor deficits.

A typical protocol for the evaluation of antibody in the APP/PS1 double transgenic mouse model for Alzheimer's disease is as follows: Treatments start at 5-6 weeks of age. Transgenic mice are randomized into 3 main groups (control and 2 doses of compound) with 10-12 animals per group. Each group is treated for up to 8 (before plaques deposition), 12 (when plaques start appearing) and 18-19 weeks of age (when amyloid burden is very pronounced), as shown above.

At each time point, the mice will be euthanized and perfused with saline or sucrose, brains will be harvested and hemispheres will be separated. One hemisphere will be fixed by immersion in parafonmaldehyde, sectioned, and stained with Campbell-Switzer Alzheimer silver stain for plaque detection, or additional staining protocols. The other hemisphere will be snap frozen and then processed for ELISA testing, for the quantification of soluble and insoluble, 1-40 and 1-42, β -amyloid.

Prior to sacrifice, behavioral testing, such as the Y-maze, "place set" Morris water maze and trace conditioning can be performed.

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WHAT IS CLAIMED IS

1. Use of an antibody which is targeted to amyloid β peptide, or to fragment thereof for the manufacturing of a medicament for treating a subject having Alzheimer's Disease.

2. Use of an antibody which is targeted to amyloid β peptide, or to fragment thereof for the manufacturing of a medicament for treating a subject having disease or disorder characterized by amyloid beta deposition.

3. Use of an antibody which is targeted to amyloid β peptide, or to fragment thereof for the manufacturing of a medicament for delaying or inhibiting or suppressing accumulation of amyloid β peptide or fragment thereof in the brain.

4. Use of an antibody which is targeted to amyloid β peptide, or to fragment thereof for the manufacturing of a medicament for delaying or inhibiting or suppressing the neurotoxicity of amyloid β peptide or fragment thereof.

5. The use according to claims 1- 4, wherein the antibody is directed to amyloid β peptide, or fragment thereof.

6. The use according to claims 1-4, wherein the antibody is directed to N-terminus-truncated amyloid β peptide fragment.

7. The use according to claims 1-4, wherein the antibody is directed to C-terminus-truncated amyloid β peptide fragment.

8. The use according to claims 1-4, wherein the antibody is directed to the amyloid precursor protein, or fragment thereof.

9. The use according to claims 1-4, wherein the antibody is a monoclonal antibody, a humanized antibody, a chimeric antibody, a bispecific antibody, an artificial antibody, a scFv antibody or a F(ab), or fragment thereof.

10. The use according to claim 2, wherein said disorder or disease characterized by amyloid beta deposition is mild cognitive impairment (MCI), cerebral amyloid angiopathy or congiophylic angiophaty, Alzheimer's Disease associated with Down Syndrome or Inclusion-body myositis.

11. An antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide.

12. An antibody that is free-end specific and is targeted to the free N-terminus of amyloid β-peptide, wherein the first amino acid of amyloid β-peptide of said is aspartate.

13. An antibody that is free-end specific and is targeted to the free N terminus of N- and/or C-terminus-truncated amyloid β peptide fragment.

14. An antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-39, Aβ1-40, Aβ1-41, or Aβ1-43.

15. An antibody that is free-end specific and is targeted to the free C-terminus of N- and/or C-terminus-truncated amyloid β peptide fragment.

16. A single chain or artificial antibody that is free-end specific and is targeted to the free C-terminus of the amyloid β-peptide Aβ1-42.

17. The antibody according to claims 11-15, wherein the antibody is a monoclonal antibody, a humanized antibody, a chimeric antibody, a bispecific antibody, an artificial antibody, a scFv antibody or a F(ab), or fragment thereof.

18. A pharmaceutical composition comprising an amount of the antibody according to any of the claims 11-16 and a pharmaceutical acceptable carrier.

19. The pharmaceutical composition of claim 18, wherein the composition is administered subcutaneously, intravenously, intradermally, intramuscularly, intraperitoneally, intracerebrally, intranasally, orally, transdermally, buccally, intra-arterially, intracranially, or intracephalically.

20. Use of an antibody according to claims 11-16, for the manufacturing of a medicament for treating a subject having Alzheimer's Disease.

21. Use of an antibody according to claims 11-16, for the manufacturing of a medicament for treating a subject having disease or disorder characterized by amyloid beta deposition.

22. Use of an antibody according to claims 11-16, for the manufacturing of a medicament for delaying or inhibiting or suppressing accumulation of amyloid β peptide or fragment thereof in the brain.

23. Use of an antibody according to claims 11-16, for the manufacturing of a medicament for delaying or inhibiting or suppressing the neurotoxicity of amyloid β peptide or fragment thereof.

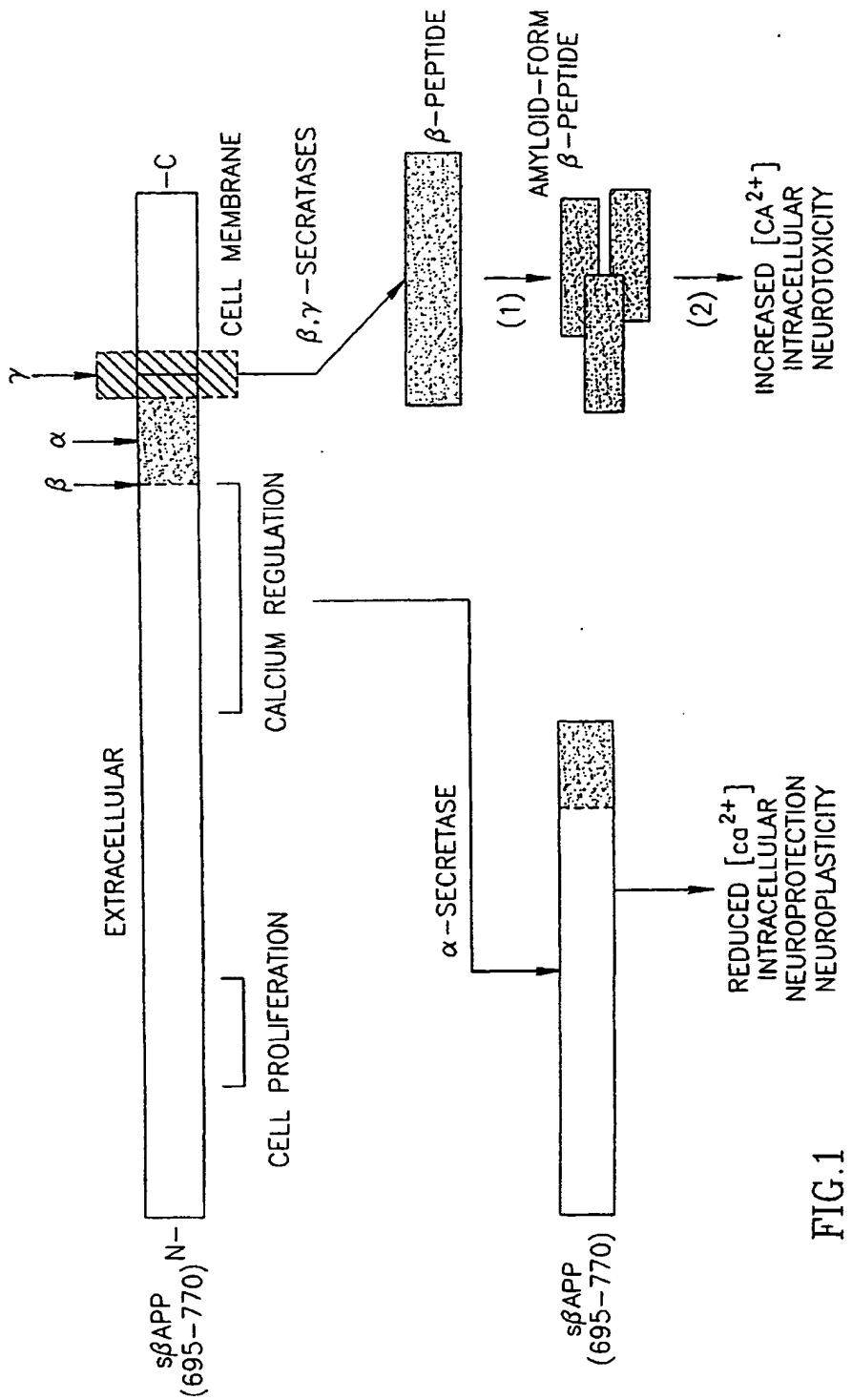

γ
α
β
EXTRACELLULAR
CELL MEMBRANE
-C
β,γ-SECRATASES
β-PEPTIDE
AMYLOID-FORM β-PEPTIDE
(1)
(2)
INCREASED [CA2+] INTRACELLULAR NEUROTOXICITY
CALCIUM REGULATION
CELL PROLIFERATION
α-SECRETASE
REDUCED [ca2+] INTRACELLULAR NEUROPROTECTION NEUROPLASTICITY
sβAPP (695-770) N-
sβAPP (695-770)

FIG.1

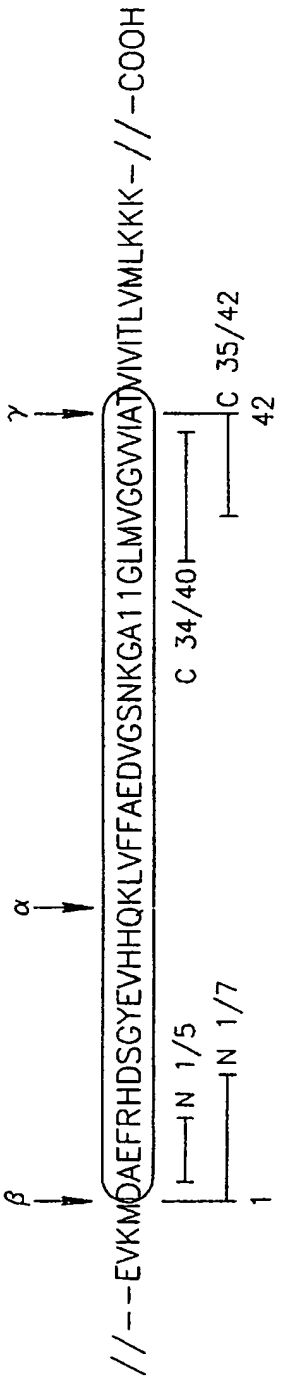

//--EVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGA11GLMVGGVVIATVIVITLVMLKKK--//-COOH
β
α
γ
N 1/5
N 1/7
1
C 34/40
C 35/42
42

FIG.2

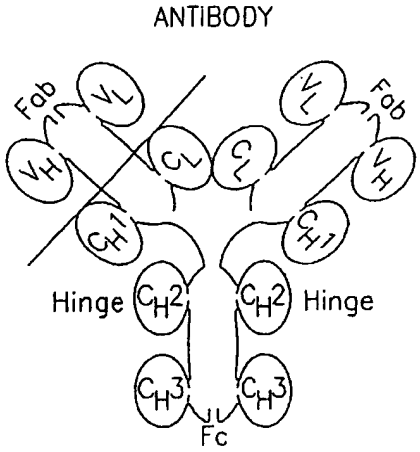


FIG.3A

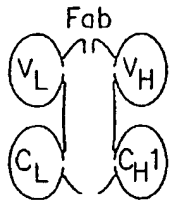


FIG.3B

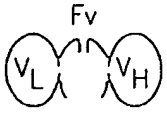
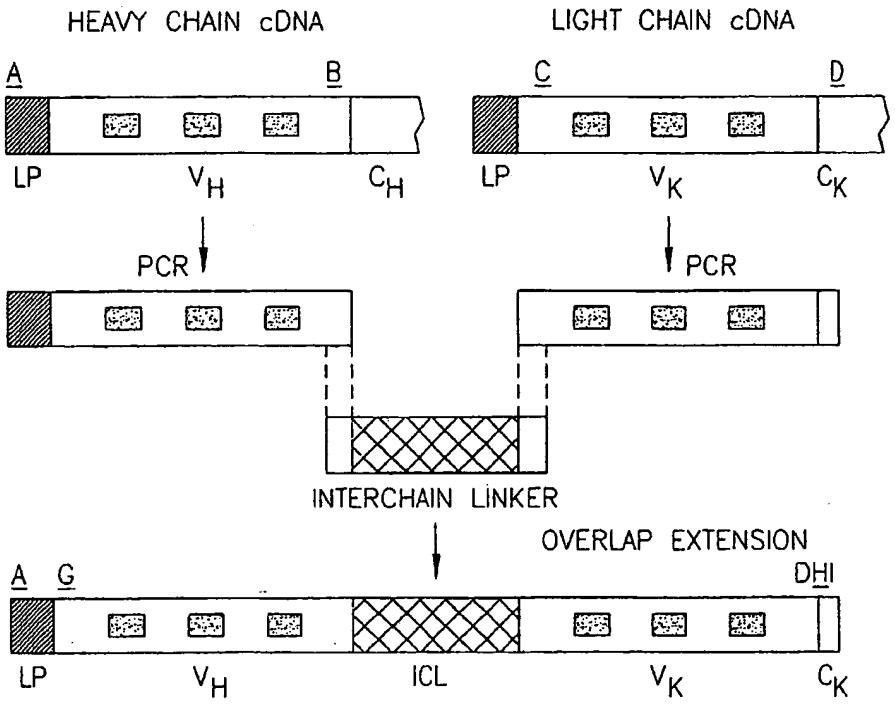


FIG.3C

SINGLE CHAIN Fv (ScFv)

$H_2N-V_H-LINKER-V_L-COOH$

FIG.3D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LIGHT CHAIN cDNA
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B
C
D
LP
V_H
C_H
LP
V_K
C_K
PCR
PCR
INTERCHAIN LINKER
OVERLAP EXTENSION
A
G
DHI
LP
V_H
ICL
V_K
C_K

FIG.4

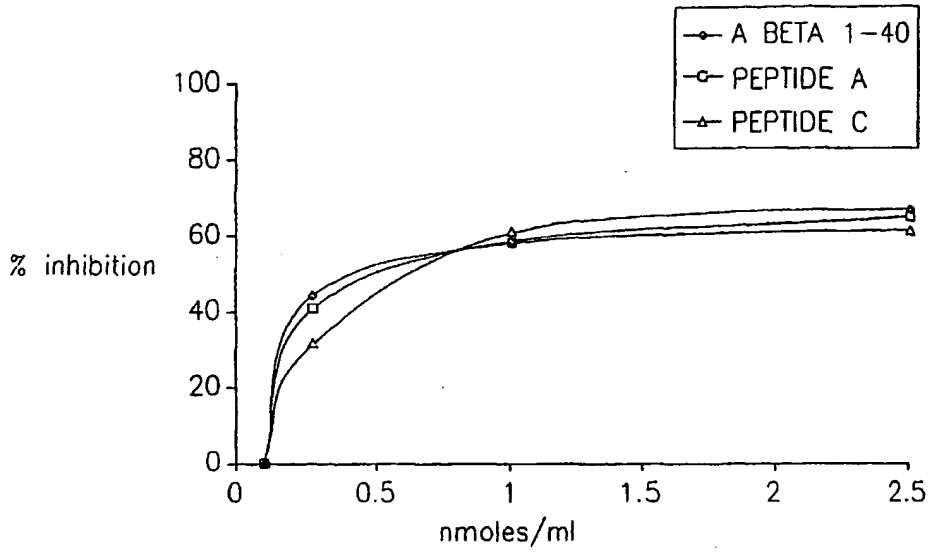
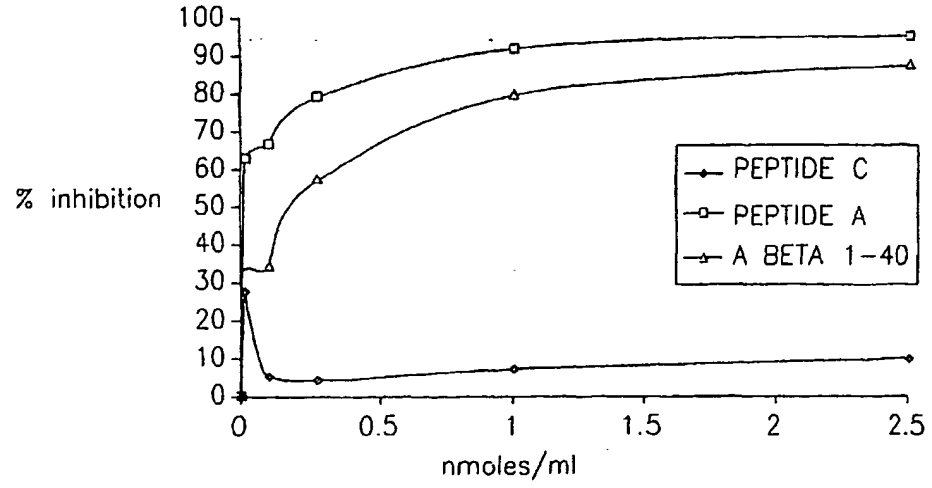

A BETA 1-40
PEPTIDE A
PEPTIDE C
% inhibition
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
nmoles/ml

FIG.5


PEPTIDE C
PEPTIDE A
A BETA 1-40
% inhibition
100
90
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70
60
50
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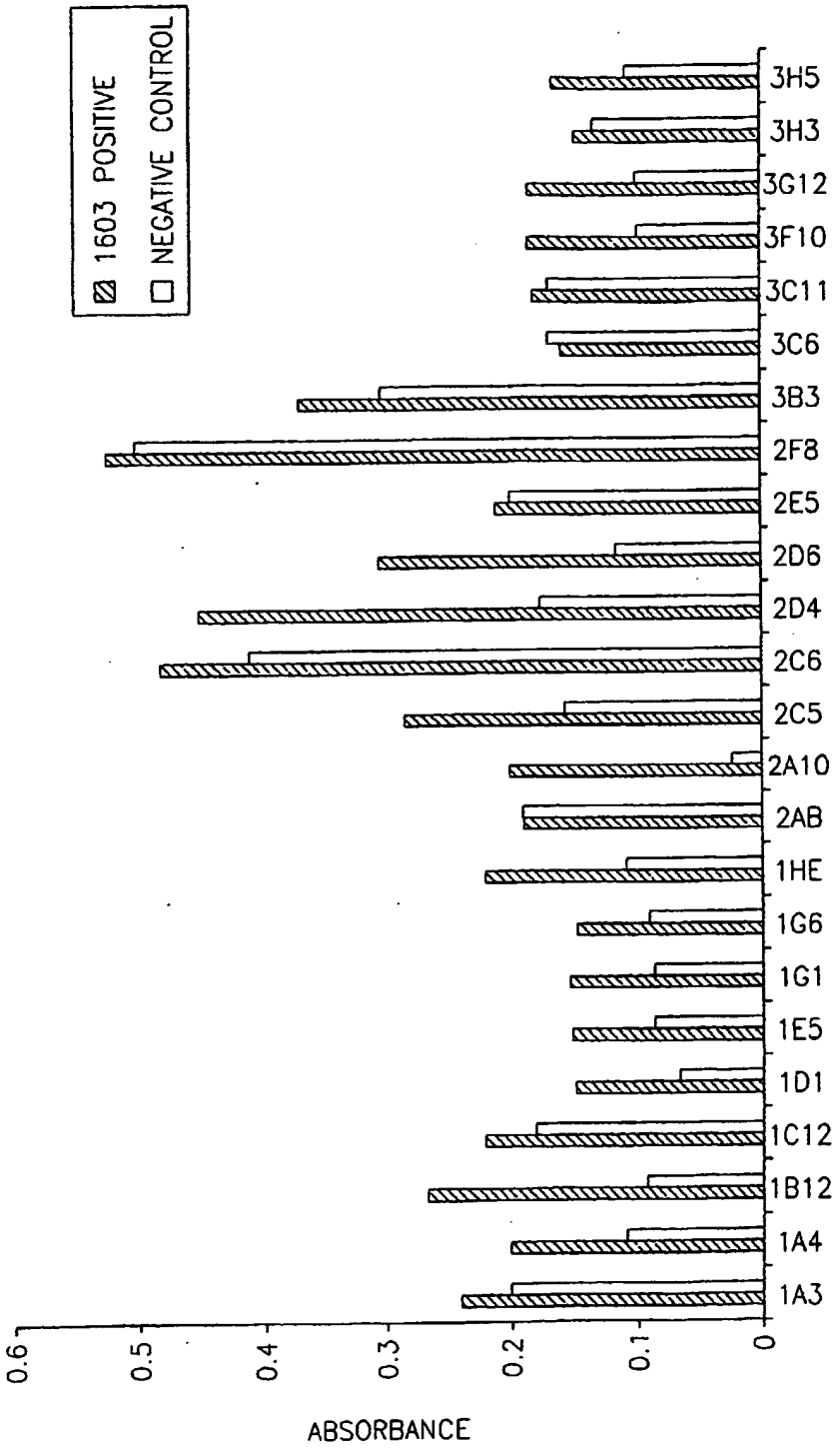
30
20
10
0
0
0.5
1
1.5
2
2.5
nmoles/ml

FIG.6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3C11
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3B3
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2D6
2D4
2C6
2C5
2A10
2AB
1HE
1G6
1G1
1E5
1D1
1C12
1B12
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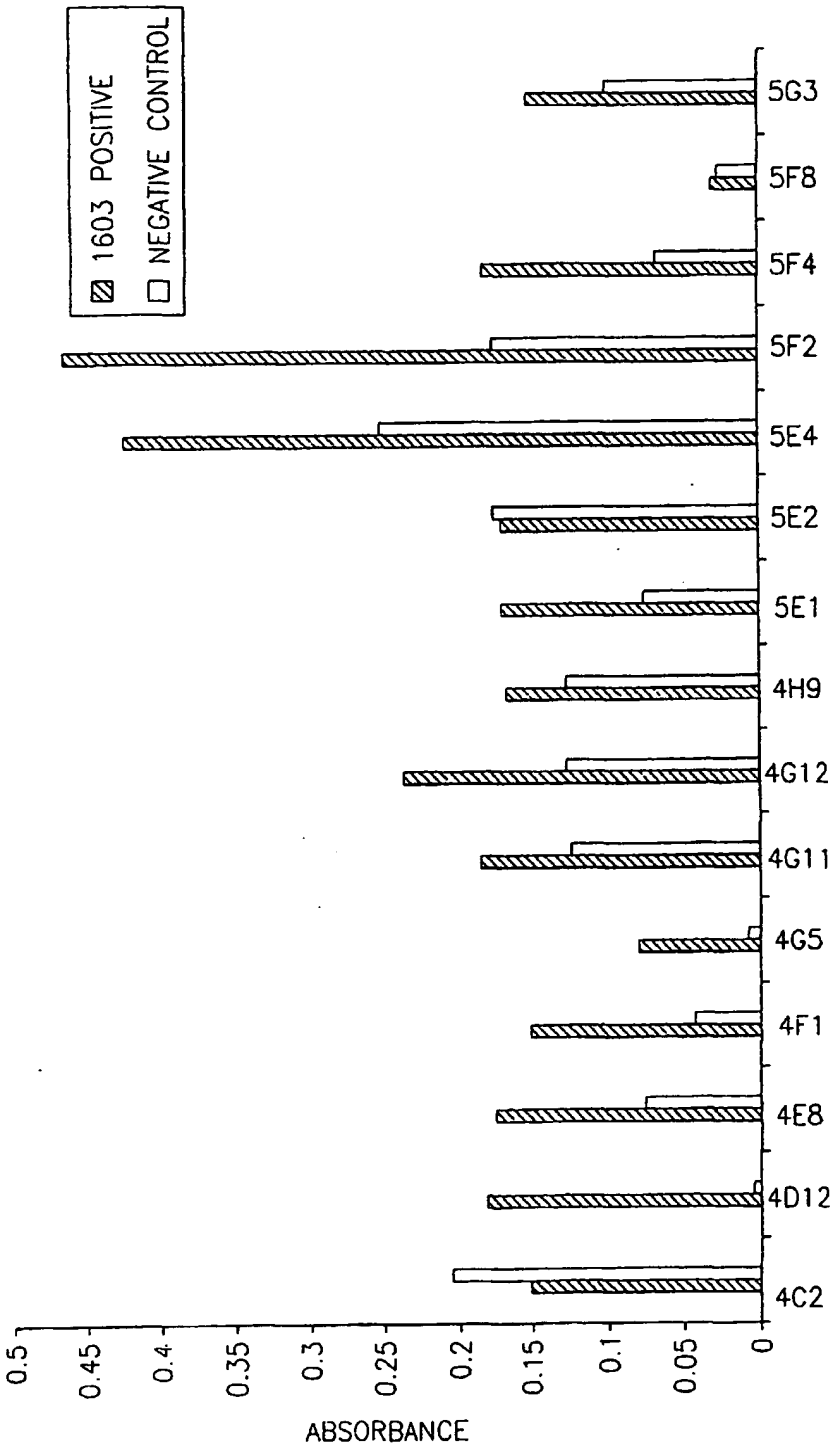
0.5
0.4
0.3
0.2
0.1
0
ABSORBANCE

FIG.7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NEGATIVE CONTROL
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5F4
5F2
5E4
5E2
5E1
4H9
4G12
4G11
4G5
4F1
4E8
4D12
4C2
0.5
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0.3
0.25
0.2
0.15
0.1
0.05
0
ABSORBANCE

FIG.8

SEQUENCE LISTING

<110> Mindset Biopharmaceutical Inc.
Mindset Biopharmaceutical Inc.
Chain, Daniel G.
Chain, Daniel G

<120> specific antibodies to amyloid beta peptide, pharmaceutical compositions and methods of use thereof

<130> P-4815PC1

<140> PCT
<141> 2002-02-28

<150> 10/084,380
<151> 2002-02-28

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<170> PatentIn version 3.1

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<213> human

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35 40 45

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys
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<210> 13
<211> 14

<212> PRT
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<400> 13

Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
1 5 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/31590

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395; C07K 16/00; C12P 21/08
US CL : 424/130.1, 133.1, 135.1; 530/387.1, 387.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/130.1, 133.1, 135.1; 530/387.1, 387.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 98/44955 A1 (MINDSET LTD.) 15 October 1998 (15.10.98) see entire document.	1-17, 19-22 --- 19-22
X --- Y	US 5,786,180 A (KONG et al) 28 July 1998 (28.07.98) see entire document.	1-17, 19-22 --- 19-22
Y	HANAN et al. Inhibitory effect of monoclonal antibodies on Alzheimer's beta-amyloid peptide aggregation. Amyloid: Int. Exp. Clin. Invest. 1996, Volumne 3, pages 130-133, see especially page 132, column 2, last two paragraphs.	1-22
Y	SOLOMON et al. Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. Proc. Nat. Acad. Sci. USA. January 1996, Volumne 93, pages 452-455, see especially page 454, column 2, last paragraph.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 2003 (13.02.2003)	21 APR 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Patricia A. Duffy Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US02/31590

Continuation of B. FIELDS SEARCHED Item 3:
WEST, MEDLINE, EMBASE, JAPIO.
SEARCH TERMS: end-specific antibodies, amyloid, beta amyloid, beta A4 amyloid, amyloid peptide, 1-40, 1-42, 1-42, recombinant, chimeric, single chain.